

RESEARCH NOTE

PHYLOGENETIC AND POPULATION GENETIC STUDY USING DYS19 FOR *ORANG ASLI* IN TAMAN NEGARA PAHANG: PRELIMINARY FINDINGS

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Orang Asli or the aborigines of Peninsular Malaysia are divided into three tribes namely Negrito (Semang), Senoi and Proto-Malay (Ang *et al.*, 2012). Y-STRs preserve a simpler record of history, compared to other recombining chromosomes (Myres *et al.*, 2009; Jain *et al.*, 2011; Parvathy *et al.*, 2012). According to phylogenetic study using maternal marker, *Orang Asli* was grouped in the same cluster with high bootstrap value (Ang *et al.*, 2012). The objective of this preliminary study was to calculate the heterozygosity and diversity of *Orang Asli* population in Taman Negara, Pahang based on paternal marker, Y-STR (DYS19) marker. The data obtained in this study will be used as comparison for further study using other markers of Y-STR.

Orang Asli samples from villages in and around Taman Negara Pahang namely Kuala Atok, Kampung Dedari and Sungai Tiang were collected with approval obtained from the Research Management Institute (RMI) of Universiti Teknologi MARA (UiTM) and *Jabatan Kemajuan Orang Asli* (JAKOA). Informed consent was obtained from each individual. The buccal swab was obtained and applied on FTA card.

The FTA cards were punched into small discs and washed with FTA purification reagent and TE buffer. The discs were transferred into Q5 High Fidelity PCR Kit (New England Biolabs) together with DYS19 marker. Polymerase chain reactions (PCR) were performed using a thermocycler (Takara). From 49 samples taken, 24 were successfully amplified which are nine, three and twelve samples for Kuala Atok, Kampung Dedari and Sungai Tiang respectively. Presence of PCR products were detected by 2% agarose gel electrophoresis and

visualized under the UV ray. Samples with present bands were sent for DNA sequencing (MyTACG Bioscience Enterprise) for further analysis.

The pairwise genetic distance was calculated using the Kimura 2-parameter model, $\hat{d} = -\frac{1}{2} \ln(1 - 2p - q) - \frac{1}{4} \ln(1 - 2q)$ where p and q represents the fraction of sites showing transitional and transversional differences respectively (Kimura, 1980). The heterozygosity within the population of Kampung Kuala Atok, Kampung Sungai Tiang and Kampung Dedari were calculated based on Nei's formula $\xi = 1 - \sum p_i^2$ where p_i represents the frequency of i th allele in a population (Nei, 1978).

Fixation index, $F_{ST} = \frac{H_T - H_S}{H_T}$ where H_T and H_S are the expected heterozygosity in random mating total population and subpopulation respectively (Wright, 1951). The phylogenetic tree was constructed using software MEGA 5.2 neighbour-joining (NJ) method. The tree was subjected to 500 bootstrap replicates. Amerindian sequence was chosen as the out-group. The pairwise genetic distance values obtained ranged from 0.011 to 0.253 while the overall mean distance was 0.090. This shows that, there are few differences in the Y-STR composition in these subjects although coming from different villages and sub tribes. However, more markers and larger sample size are required to conclude that finding. Heterozygosity of the sample population was 0.3424 indicating that the diversity among the subjects from different villages in Taman Negara was low. F_{ST} , a measure of population differentiation due to genetic structure was calculated and gave a value of 0.0457 meaning that 4.57% of the variation was found within sub tribes while 95.43% variation was found distributed among all sample population. This shows relatively very low diversity of the subjects in a sub tribes or a sample population represented by 4.57%

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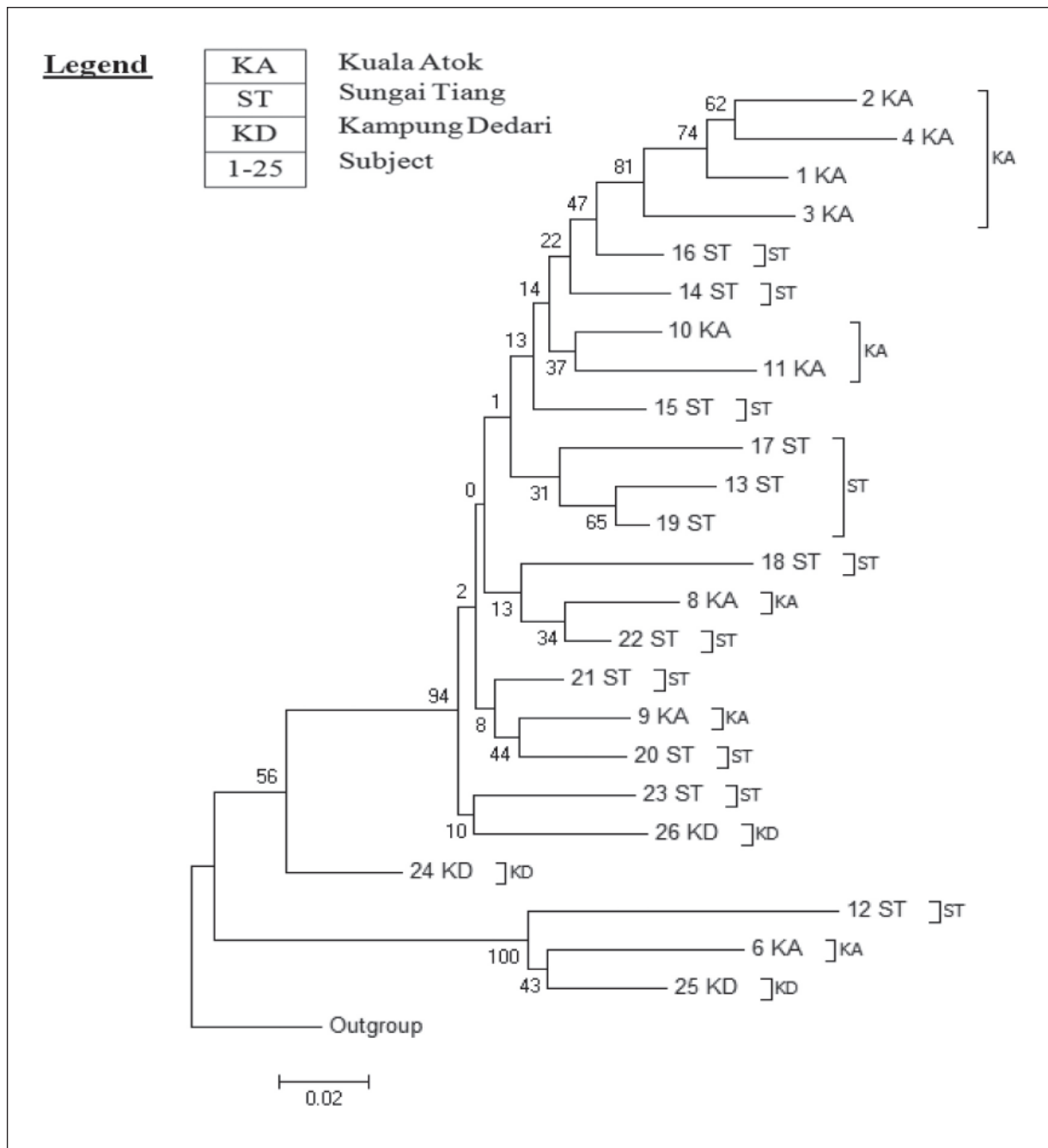


Fig. 1. Phylogram built from NJ analysis.

of total diversity compared to 95.43% found in the total population. This finding is agreement to the hypothesis that subjects in a small, isolated population are less diverse in terms of genetic composition compared to subjects

A phylogenetic tree was constructed using the neighbor-joining (NJ) method as shown in Fig. 1. The grouping of subjects according to tribes can be seen. For example, sample 1, 2, 3 and 4 from Kampung Atok and subject 13, 17 and 19 from Sungai Tiang are in the same subclade. However,

there are clades made up of subjects from different tribes, for example subject 23 from Sungai Tiang with subject 26 from Kampung Dedari. Low bootstrap values of such clades indicate less resolution in tree topology, thus the relationships are not well supported.

In conclusion, more markers and samples are required to gain better insight on the genetic composition of a population. More samples and larger area of coverage will provide more accurate information.

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